

EVALUATION OF A NEW SPRAYING MACHINE FOR BARRIER TREATMENT AND PENETRATION OF BIFENTHRIN ON VEGETATION AGAINST MOSQUITOES

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ABSTRACT. The effectiveness and penetration of a novel, truck-mounted mist sprayer (3WC-30-4P provided by American LongRay) was evaluated with bifenthrin in a large, park-like setting with historic floodwater and woodland mosquito populations. Efficacy evaluations were determined through adult population collections and excised leaf bioassays. Trapping results showed a mean reduction of 77% in mosquito populations in the treated area for 5 sampling events up to 4 wk posttreatment. Leaf bioassays revealed an average mortality of 80% at 2.7 m and 51% at 5.5 m against laboratory-reared *Aedes aegypti* for 5 posttreatment samples. Leaves collected from the treated areas caused higher mortality at distances closer to the sprayer, though the distance and coverage of bifenthrin application was effective up to 5 m.

KEY WORDS Mist spray machine, barrier treatment, bifenthrin, *Aedes aegypti*, vegetation

INTRODUCTION

Many species of adult mosquitoes utilize the structures of plants for a variety of purposes, from resting to nutrition, depending on the mosquito species (Xue 2008). Applications of insecticides to plant foliage are an effective tool in operational mosquito control (Amoo et al. 2008, Xue 2008, Qualls et al. 2013) and can provide cost savings when analyzing service requests from residents (Qualls et al. 2012). Effective barrier treatments will enable mosquito–insecticide contact during resting or sugar-feeding periods. The goals of barrier treatments include reducing broad-scale application of chemicals (Royal 2004) and stopping adult mosquitoes from entering untreated outdoor areas used for human recreation (Cilek and Hallmon 2006, 2008; Cilek 2008).

Previous research illustrated the effectiveness of hand-pump and petroleum-powered backpack sprayers for barrier treatment application (Amoo et al. 2008), focusing on residential areas (Hubbard et al. 2005, Trout et al. 2007, Li et al. 2010). Several types of powered application devices and other technologies, including mist blowers, retrofitted pressure washers, electrostatic and conventional sprayers, such as ultra-low volume, have

been used to apply bifenthrin for mosquito control barrier treatments (Allan et al. 2009, Britch et al. 2009, Hoffmann et al. 2009, Farooq et al. 2010, Qualls et al. 2013). Barrier treatments with the pyrethroid bifenthrin and other residual adulticides have been successful in controlling several vector and nuisance mosquito species for multiple weeks (Trout et al. 2007; Amoo et al. 2008; Cilek 2008; Xue 2008; Doyle et al. 2009; Qualls et al. 2012, 2013). The density of vegetation has proven to be challenging for mosquito control using electrostatic sprayers in dense vegetation (Anderson et al. 1991, Farooq et al. 2010). For barrier treatments, it is crucial that the equipment push the insecticide droplets onto the vegetation. For mosquito control programs that must target large areas efficiently, truck-mounted barrier application equipment has the potential for operational timesaving, providing deeper coverage sufficient to effectively control multiple mosquito populations inhabiting dense vegetation.

Our study evaluated the posttreatment residual effectiveness of bifenthrin applied by a new truck-mounted mist sprayer with 4 nozzles against the natural population of adult mosquitoes, through field-site adult mosquito trapping and laboratory leaf bioassays. Here we report on the efficacy and penetration of a bifenthrin barrier application in a simulated large, park-like setting.

MATERIALS AND METHODS

Study area

The study was conducted on a vegetated perimeter at the Anastasia Mosquito Control District's (AMCD's) 7.3-ha property (29°54'09.32"N, 81°24'47.12"W) from October 4 to November 9, 2013 (Fig. 1). The area was chosen because of its large interior park-like setting, surrounding dense

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| Report Documentation Page | | | Form Approved OMB No. 0704-0188 | | |
|--|------------------------------------|-------------------------------------|---|---|---------------------------------|
| Public reporting burden for the collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to a penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. | | | | | |
| 1. REPORT DATE MAR 2015 | | 2. REPORT TYPE | | 3. DATES COVERED 00-00-2015 to 00-00-2015 | |
| 4. TITLE AND SUBTITLE Evaluation of a New Spraying Machine for Barrier Treatment and Penetration of Bifenthrin on Vegetation Against Mosquitoes | | | 5a. CONTRACT NUMBER | | |
| | | | 5b. GRANT NUMBER | | |
| | | | 5c. PROGRAM ELEMENT NUMBER | | |
| 6. AUTHOR(S) | | | 5d. PROJECT NUMBER | | |
| | | | 5e. TASK NUMBER | | |
| | | | 5f. WORK UNIT NUMBER | | |
| 7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Navy Entomology Center of Excellence,Naval Air Station,PO Box 43,Jacksonville,FL,32212 | | | 8. PERFORMING ORGANIZATION REPORT NUMBER | | |
| 9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) | | | 10. SPONSOR/MONITOR'S ACRONYM(S) | | |
| | | | 11. SPONSOR/MONITOR'S REPORT NUMBER(S) | | |
| 12. DISTRIBUTION/AVAILABILITY STATEMENT Approved for public release; distribution unlimited | | | | | |
| 13. SUPPLEMENTARY NOTES | | | | | |
| 14. ABSTRACT like setting with historic floodwater and woodland mosquito populations. Efficacy evaluations were determined through adult population collections and excised leaf bioassays. Trapping results showed a mean reduction of 77% in mosquito populations in the treated area for 5 sampling events up to 4 wk posttreatment. Leaf bioassays revealed an average mortality of 80% at 2.7 m and 51% at 5.5 m against laboratory-reared Aedes aegypti for 5 posttreatment samples. Leaves collected from the treated areas caused higher mortality at distances closer to the sprayer, though the distance and coverage of bifenthrin application was effective up to 5 m. | | | | | |
| 15. SUBJECT TERMS | | | | | |
| 16. SECURITY CLASSIFICATION OF: | | | 17. LIMITATION OF ABSTRACT Same as Report (SAR) | 18. NUMBER OF PAGES 8 | 19a. NAME OF RESPONSIBLE PERSON |
| a. REPORT unclassified | b. ABSTRACT unclassified | c. THIS PAGE unclassified | | | |

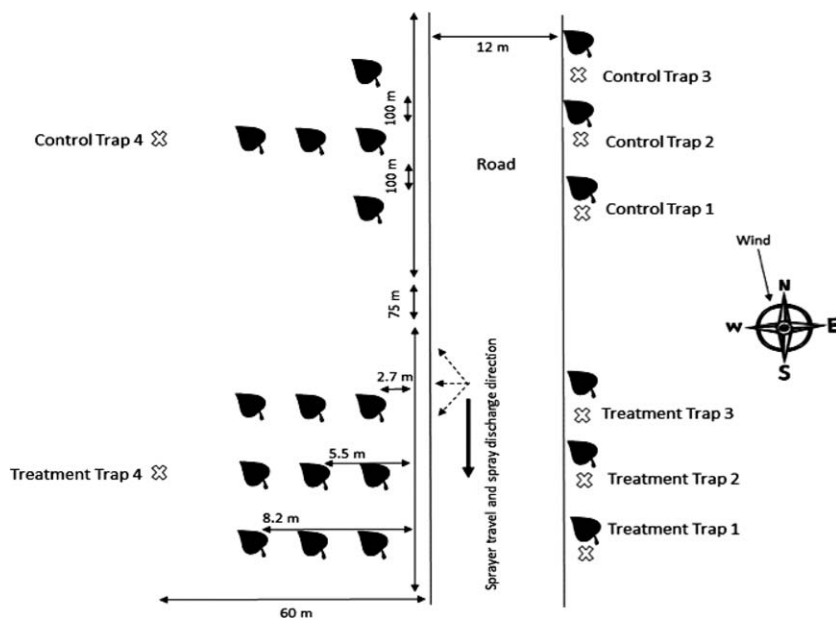


Fig. 1. Overview of treatment and control Centers for Disease Control and Prevention (CDC) trapping and leaf bioassay plots showing trapping sites, sampling line distances, and sampling sites.

vegetation with similar habitats, suitable environments for control and treatment sites, and both variety and density of mosquito species historically present. A general description of the treated and control area vegetation that surrounded the 7.3-ha property follows: Pines (*Pinus* spp.), American sweetgum (*Liquidambar styraciflua* L.), and red maple (*Acer rubrum* L.) dominated upper and midstories while a thick understory of sweetbay magnolia (*Magnolia virginiana* L.), wax myrtle (*Morella cerifera* L.), and multiple vine species, including American wild grape (*Vitis rotundifolia* Michx.) was present. The evergreen and deciduous vegetation was mixed around the property.

Trapping and leaf bioassay treatment and control sites were chosen for similarity in habitat, density of vegetation, and similar mixed sun and shade lighting running north to south on the west side of the property. A lowland swamp was adjacent and to the west of the treatment and control areas. This swamp has been identified as a prime habitat for floodwater mosquitoes (AMCD, unpublished data). Through historical trapping of adult mosquitoes using the Centers for Disease Control and Prevention (CDC) (John W. Hock, Gainesville, FL) dry ice-baited light traps, we know that *Anopheles crucians* Wiedemann, *Aedes atlanticus* Dyar and Knab, *Psorophora columbiae* Dyar and Knab, and *Culex nigripalpus* Theobald are common during the late summer and early fall when this study was carried out (AMCD, unpublished data). The entire treated barrier was approximately 3-km distance

surrounding the 7.3-ha property. The 300-m trapping and leaf bioassay sampling lengths for each of treatment and control sections had a 75-m buffer between the end of the treated area and the start of the control area. The buffer zone and control area were to the north of the treated area.

Equipment

A truck-mounted mist sprayer (model 3WC-30-4P; American LongRay, San Francisco, CA) was calibrated at the Navy Entomology Center of Excellence (NECE), Jacksonville, FL, prior to the treatment date with BVA-13 mineral oil (Adapco Inc., Sanford, FL). During calibration, the flow rate was set at 4.7 liter/min of BVA-13 oil at 3,200 revolutions/min engine speed and resulted in a median droplet diameter ($D_{v0.5}$) of 107.5 μ m and mean droplet velocity of 6.9 m/sec when measured with a 2D Phase-Doppler Particle Analyzer system (TSI Inc., Shoreview, MN). The sprayer nozzle heads were set at 360° vertically and 330° laterally in order to create a spray pattern 3 m high and 3.1 m wide, respectively, when the truck was stationary. A 68.1-liter external tank was utilized for the application.

The fogger is powered by a 6.3-kW 16-liter diesel engine. The unit has 4 adjustable spray heads. The 66 × 121 × 117-cm unit weighs 200 kg and has a 50-liter internal pesticide tank as well as a connection for an external tank. Flow rate can be continuously varied from 0.83 to 5.00 liter/min. The spray liquid is atomized by a high-speed swirling air current inside the nozzle. The engine

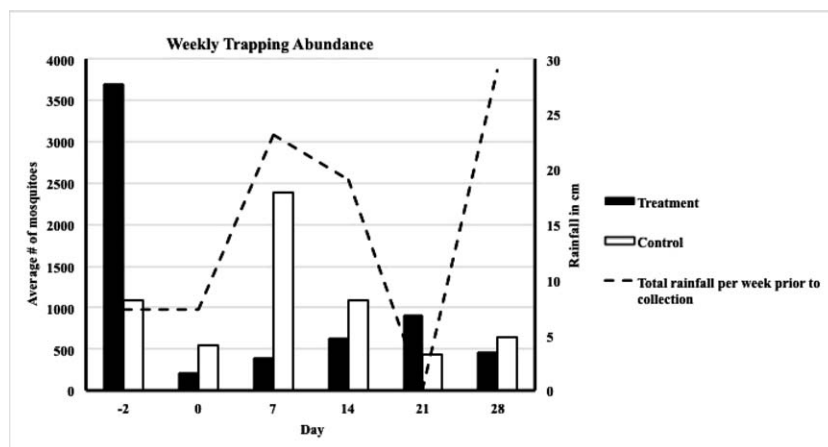


Fig. 2. Average trapping results for each day as well as total rainfall per week prior to trapping day.

can be turned on and off using a remote control up to 5.5 m away from the sprayer.

Field test

Environmental conditions were monitored with a handheld anemometer (Kestrel 1000, Boothwyn, PA) and cross-referenced with National Oceanic and Atmospheric Administration (NOAA) weather data collected at the St. Augustine Airport, 9.6 km NE from the spray site. Wind speed at the time of application averaged 1.3 m/sec according to the anemometer and 3.1 m/sec NNW according to NOAA. Spray from the bifenthrin application did not drift into the control area due to the wind direction. No precipitation was recorded on the day of application. The RH averaged 90% and the ambient temperature was 25.6°C at the time of application.

Insecticide application: The barrier treatment consisted of bifenthrin (TalstarP®, AI 7.9%; FMC Corporation, Philadelphia, PA) diluted to 7.4 ml of formulation per liter of well water and applied at the label rate of 41 ml/m², using the aforementioned truck-mounted sprayer at a driving speed averaging 4.7 km/h. The application rate resulted in 0.76 g AI/m² of the vegetation surface. For the whole treatment plot, 1.2 liters of formulation mixed with 162 liters of well water was used to cover the 3.2 km of vegetation. During the treatment, the ends of the nozzles of the machine were approximately 2.2 m from the nearest vegetation. The control plot was untreated. Runoff of insecticide was not observed.

Mosquito monitoring: Weekly, lighted CDC light traps baited with approximately 1.8 kg dry ice in a 3.8-liter Igloo® Legend Beverage Cooler (Wal-Mart, St. Augustine, FL) with holes for gas release were placed at the same locations on shepherd's hooks in the field at 0800 h and collected after 24 h (Fig. 1). The treatment and

control trapping areas each had 3 traps that were separated by 100 m for a total of 6 CDC traps east of the barrier spray. These traps were placed approximately 12 m east of the barrier spray zone to encourage the floodwater species developing to the west to come through the control and treatment areas towards the traps. The distance between the closest treatment and control traps was 360 m. In addition, 2 CDC light traps baited with 1-octen-3-ol (BioSensory Inc., Willimantic, CT) were placed within the floodwater mosquito developmental areas west of the barrier treatment and control areas. Treatment trap number 4 was centered along the length of the treated area while control trap number 4 was centered along the length of the control area, each approximately 61 m west of the treatment zone to monitor the floodwater breeding sites to establish source mosquito population (Fig. 1).

In the 6 CDC light traps, pretreatment mosquito populations were sampled 2 days prior to the insecticide application. Sampling occurred 0, 7, 14, 21, and 28 days posttreatment. All mosquito collections were returned to the laboratory for identification to species. Average rainfall was monitored throughout the study period from nearby AMCD rain gauge data and NOAA, respectively (Fig. 2).

Laboratory leaf bioassay

In order to confirm residual efficacy of bifenthrin throughout the canopy and over time, leaf bioassays were conducted in the laboratory. Leaf bioassay sampling consisted of 3 sampling lines in both the bifenthrin-treated and untreated sections, providing a total of 6 sampling lines (Fig. 1). Along the line, leaf sampling sites were marked before bifenthrin application, enabling weekly leaf collection from the same vegetation in both the treated and untreated areas. Following

recovery of CDC light trap collections, leaves were removed from plants and returned to the laboratory for bioassay procedures. Weekly sampling times averaged 1.5 h.

From the literature we know that certain plant species with new growth could impact the effectiveness of bifenthrin (Doyle et al. 2009); therefore, we collected samples from deciduous vegetation and only selected leaves that were present on the treatment date. Leaves from 3 species of plants were used for this evaluation: American sweetgum, American wild grape, and red maple. On each sampling date, leaves were picked from the same tree or vine at similar heights or within 0.3 m of the marked vegetation, choosing leaves of similar size. On each sampling line, leaves were collected at each of the marked sites from heights averaging 1.5 m (range: 1.0–2.1 m) at distances of 2.7, 5.5, 8.2, and –12 m from the spray nozzle (Fig. 1) and placed individually in labeled sealable bags. The –12-m distance was used to monitor potential drift of the chemical in the opposite direction from the treatment area. Nitrile gloves were changed and clipping shears were cleaned with 70% isopropyl alcohol-soaked cotton balls between every leaf collected. Collected samples were stored in dark-colored Rubbermaid® (Winchester, VA) tubs following collection and during transport back to the AMCD laboratory to minimize photo-degradation.

Two sets of bioassays were conducted for each sampling day during the study period: one at AMCD laboratory and one at the University of Florida Veterinary Entomology Laboratory (UFVEL). Leaf samples for both laboratories were collected together. All samples were taken to the AMCD laboratory and either frozen for the bioassay at UFVEL or immediately utilized at AMCD.

While researchers were wearing gloves, leaves were placed top side up in a large petri dish (150 × 25 mm; Fisher Scientific, Atlanta, GA). At the UFVEL, mosquitoes were anesthetized by CO₂ then placed into petri dishes, whereas AMCD personnel gently aspirated mosquitoes (*Aedes aegypti* (L.)) by mouth into the petri dishes. All CO₂-exposed mosquitoes were monitored for recovery and replaced if found nonresponsive. Petri dishes had holes in the top sides for aspirating and for the placement of a 10% sugar solution-soaked cotton ball. Not all leaves flattened in the petri dishes, allowing mosquito adults access to undersides of leaves. For each bioassay, 1 leaf was placed into a petri dish, with 4 leaves per sampling line, yielding 12 petri dishes for the bifenthrin treatment and 8 for the untreated control area.

Mosquitoes were obtained from a colony at the UFVEL, where they have been maintained for >30 years without insecticide exposure. Following adult emergence, mixed-sex mosquitoes were

maintained on a 10% sugar solution in the insectary (12L:12D, 26.6°C, 60% RH). Ten adult female *Ae. aegypti* (average 9 days old) were placed in a petri dish. Treatment and controls were held in separate incubators (Precision, Winchester, VA) with a 12L:12D photoperiod at 26.6°C. Mortality was recorded after 24 h. Individuals unable to stand were counted as dead.

The area of the leaves sampled was calculated using a Li 3050C Transparent Conveyor Accessory and Li 3000C Portable Area Meter (Li-Cor Biosciences, Lincoln, NE) at the NECE. American sweetgum leaf areas averaged 50.09 cm², American wild grape 45.23 cm², and red maple 37.97 cm².

Data analysis

The mosquito collections in 3 control and 3 treatment traps were averaged and analyzed for posttreatment percent population reductions and for species compared with the pretreatment mosquito collection means. In order to determine the percentage of weekly population reduction compared with the pretreatment, we utilized Mulla's formula (Mulla et al. 1971): % reduction = $100 - (C_1/T_1 \times T_2/C_2) \times 100$. The C₁ variable was the mean number of mosquitoes from the control site traps pretreatment and C₂ was the mean number of mosquitoes from the control sites posttreatment. The T₁ variable was the mean number of mosquitoes from the treatment sites pretreatment, while T₂ was the mean number of mosquitoes from the treatment sites posttreatment.

For the leaf bioassay, statistical analysis was performed using JMP 11.1 software (SAS Institute Inc., Cary, NC). Treatment mortality was corrected with Abbott's formula (Abbott 1925) prior to statistical analysis using the same-day mortality values obtained from corresponding distance values in the control plots. Mortality data for the 2 leaf bioassays were pooled and averaged, as no significant differences were found between bioassays in the 2 labs. The ANOVA was used with alpha (α) = 0.05 for mortality comparison within and between each distance, using Tukey's mean separation. The data were found to be nonnormal even after transformation. Both ANOVA and Kruskal-Wallis procedures provided similar results regarding significant differences and means comparisons; therefore, the results of ANOVA were reported. Pretreatment leaf bioassays were not included in the analysis due to the high control mortality and only a single pretreatment evaluation.

RESULTS

Based on the CDC light trap data, the bifenthrin barrier application reduced the natural

Table 1. Average adult mosquito populations in lighted and dry ice-baited Centers for Disease Control and Prevention light traps collected pre- and posttreatment, St. Augustine, FL.

| | Pretreatment (days) | | Posttreatment (days) | | | |
|--------------------------------|---------------------|-----|----------------------|-------|-----|-----|
| Average populations | −2 | 0 | 7 | 14 | 21 | 28 |
| Control | 1,092 | 552 | 2,390 | 1,086 | 431 | 640 |
| Treatment | 3,691 | 208 | 396 | 629 | 914 | 457 |
| Percent reduction ¹ | | 89 | 95 | 83 | 37 | 79 |
| Treatment | | | | | | |

¹ Percent reduction formula from Mulla et al. (1971).

mosquito populations by 89%, 95%, 83%, 37%, and 79% on day 0, 7, 14, 21, and 28 posttreatment, respectively (Table 1). There was no significant difference between pre- and posttreatment trapping in the untreated area ($F_{1,5} = 0.007$, $P > 0.05$). Significant differences were observed between pre- and posttreatment trapping of mosquitoes within the bifenthrin-treated area ($F_{1,5} = 118.17$, $P < 0.001$). There was no significant difference between the overall mosquito captures from traps placed between the barrier spray and the floodwater breeding area ($F_{1,11} = 0.0016$, $P > 0.05$); therefore, immigration-source mosquito populations stayed consistent when comparing the treatment and control areas. Thirteen mosquito species from 7 genera were collected from all treatments and controls in the CDC light traps used during the study period. The majority of traps were comprised of *An. crucians*, followed by *Cx. nigripalpus*, *Ae. infirmatus* Dyar and Knab, and *Coquillettidia perturbans* Walker. Other species included *An. quadrimaculatus* Say, *Culiseta melanura* Coquillett, *Cx. coronator* Dyar and Knab, *Cx. erraticus* Dyar and Knab, *Ps. ciliata* Fabricius, *Ps. columbiae*, *Ps. ferox* von Humboldt, and *Uranotaenia sapphirina* Osten Sacken (Table 2).

The difference between average mortality of treatments and controls in the leaf bioassay was statistically significant ($F_{1,99} = 24$, $P = < 0.001$). There was no significant difference in mortality between distances in the control areas ($F_{3,39} = 0.86$, $P > 0.05$) and between sampling lines ($F_{2,59} = 2.99$, $P = 0.05$). In the treatment area, the difference in mortality was highly significant between the different sample distances ($F_{3,59} = 20.11$, $P < 0.001$) (Table 3). Mortality in the treated area at an average distance of 2.7 m from the spray nozzle ranged from 98.3% at 1 wk to 61.6% at 4 wk posttreatment (Fig. 3). Similarly, at a distance of 5.5 m, mosquito mortality was reduced from 71.3% at 1 wk down to 31.1% on 4 wk posttreatment. At distances of 8.2 and −12 m, mortality from 30% after 1 wk posttreatment went down to 0 after 4 wk posttreatment. Leaf bioassays against *Ae. aegypti* adults revealed a mean mortality of 80% at 2.7 m, 51% at 5.5 m, 6.4% at 8.2 m, and 7.2% at −12 m.

DISCUSSION

Barrier treatments by the AMCD have increased since 2008 (Qualls et al. 2013). Because mosquitoes can come from a variety of habitats

Table 2. Species, total composition, and percent reduction by sampling day posttreatment of adult female mosquitoes collected by Centers for Disease Control and Prevention light traps baited with dry ice throughout the study period.¹

| | Species composition (%) | | Percent reduction | | | | |
|----------------------------------|-------------------------|---------|-------------------|-------|--------|--------|--------|
| | Treatment | Control | Day 0 | Day 7 | Day 14 | Day 21 | Day 28 |
| <i>Aedes atlanticus</i> | 2.4 | 6.7 | 97.5 | 98.7 | 94.5 | 100 | 54.8 |
| <i>Ae. infirmatus</i> | 0.8 | 0.6 | 100 | 100 | 0 | 100 | 85.1 |
| <i>Anopheles crucians</i> | 89.7 | 80.0 | 83.6 | 94.5 | 81.4 | 33.4 | 79 |
| <i>An. quadrimaculatus</i> | 0.1 | 0.0 | 0 | 0 | 0 | 0 | 0 |
| <i>Coquillettidia perturbans</i> | 0.8 | 2.8 | 98.2 | 98.4 | 92.9 | 84.4 | 70.6 |
| <i>Culiseta melanura</i> | 0.8 | 1.1 | 95 | 86.1 | 86.9 | 68.5 | 92.1 |
| <i>Culex coronator</i> | 0.0 | 0.6 | 0 | 0 | 0 | 0 | 0 |
| <i>Cx. erraticus</i> | 0.2 | 1.4 | 0 | 0 | 0 | 0 | 0 |
| <i>Cx. nigripalpus</i> | 3.1 | 6.1 | 92.7 | 97.8 | 87.5 | 72.6 | 76.9 |
| <i>Psorophora ciliata</i> | 0.1 | 0.1 | 100 | 0 | 0 | 0 | 0 |
| <i>Ps. columbiae</i> | 1.7 | 0.5 | 0 | 85 | 0 | 0 | 92 |
| <i>Ps. ferox</i> | 0.2 | 0.1 | 0 | 100 | 0 | 0 | 0 |
| <i>Uranotaenia sapphirina</i> | 0.1 | 0.1 | 0 | 0 | 0 | 0 | 0 |
| Totals | 100.0 | | 88.8 | 95.1 | 82.9 | 37.3 | 78.8 |

¹ Pooled trapping abundance by species (%). Percent reduction calculated using Mulla's formula (Mulla et al. 1971).

Table 3. Corrected mean percent mortality of adult mosquitoes by distance and sample week from leaf bioassay dates in treated and control areas.

| Week | Control distance (m) | | | | Treated distance (m) | | | |
|-------|-----------------------------|-----------|------------|-----------|--------------------------------|-------------|-------------|-------------|
| | 2.7 | 5.5 | 8.2 | -12 | 2.7 | 5.5 | 8.2 | -12 |
| 0 | 8.0 ± 2.9 | 0.0 ± 5.0 | 0.0 ± 5.0 | 4.3 ± 2.9 | 98.3 ± 1.7 | 71.3 ± 28.7 | 29.6 ± 18.9 | 30.0 ± 27.2 |
| 1 | 0.0 ± 0.0 | 0.0 ± 0.0 | 22.5 ± 0.0 | 0.0 ± 0.0 | 98.3 ± 1.7 | 60.4 ± 29.5 | 0.0 ± 0.0 | 0.6 ± 0.6 |
| 2 | 2.8 ± 1.6 | 0.0 ± 2.7 | 0.0 ± 2.7 | 1.7 ± 1.6 | 65.0 ± 32.5 | 44.4 ± 25.1 | 2.3 ± 1.2 | 2.8 ± 2.8 |
| 3 | 1.7 ± 1.7 | 0.0 ± 2.9 | 0.0 ± 2.9 | 1.7 ± 1.7 | 78.1 ± 14.4 | 48.1 ± 27.4 | 0.0 ± 0.0 | 2.6 ± 2.6 |
| 4 | 0.0 ± 1.2 | 0.0 ± 2.0 | 0.0 ± 2.0 | 1.7 ± 1.2 | 61.6 ± 30.9 | 31.1 ± 29.4 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| Total | 2.5 ± 1.2 | 0.0 ± 2.0 | 4.5 ± 2.0 | 1.9 ± 1.2 | 80.2 ± 9.0 | 51.1 ± 11.2 | 6.4 ± 4.5 | 7.2 ± 5.6 |
| ANOVA | $F_{3,39} = 0.86, P = 0.47$ | | | | $F_{3,59} = 20.11, P < 0.0001$ | | | |

and seek hosts and/or sugar sources at different times of the day, operational control requires additional equipment and product that is efficient, effective, residual, and targeted to protect humans from pest and adult vector mosquitoes (Hubbard et al. 2005). The machine evaluated here provided effective coverage of the bifenthrin formulation on barrier vegetation, with an additional benefit of effectively pushing the spray deposition up to 5.5 m into the vegetation, thus providing control of resting and sugar-feeding mosquitoes of multiple species. In addition, the combination of droplet size, 4 nozzles, and the velocity of spray from the machine factored into an effective coverage and penetration of the chemical into dense vegetation. Trapping results from pretreatment to posttreatment suggest that barrier treatment significantly reduced mosquito populations in treated areas for up to 4 wk, compared to the control. Additionally, leaf bioassays confirmed the efficacy and persistence of the bifenthrin treatment against adult mosquitoes out to 5.5 m from the nozzle and into dense vegetation compared to the controls and distances up to 8.2 m, despite the heavy rain experienced during the study period. The results from the

distance to monitor drift approximately -12 m were slightly higher than the farthest distance average mortality recorded, which will require further evaluation and testing.

Though our trapping surveillance favored crepuscular species, we trapped a variety of *Ae. atlanticus* and *Ae. infirmatus* that rest in low-lying vegetation and are pest species with diurnal host-seeking activity. Crepuscular and nocturnal host-seeking species and vectors of eastern equine encephalitis, such as *Cs. melanura* and *Cq. perturbans*, populations were significantly reduced in this study. There was a spike in treatment trap counts at 21 days posttreatment as compared with the control traps. This spike primarily was due to an increase in *An. crucians* populations, an observation that will require further evaluation and analysis.

The capability of the machine evaluated in this study to deliver a residual application of bifenthrin to the leaves in the vegetation barrier was similar to Cilek (2008) and Trout et al. (2007) for park and residential areas in which excised leaves showed >70% mosquito mortality in the laboratory up to 4 wk postapplication. Future experiments with the same chemical and equipment will

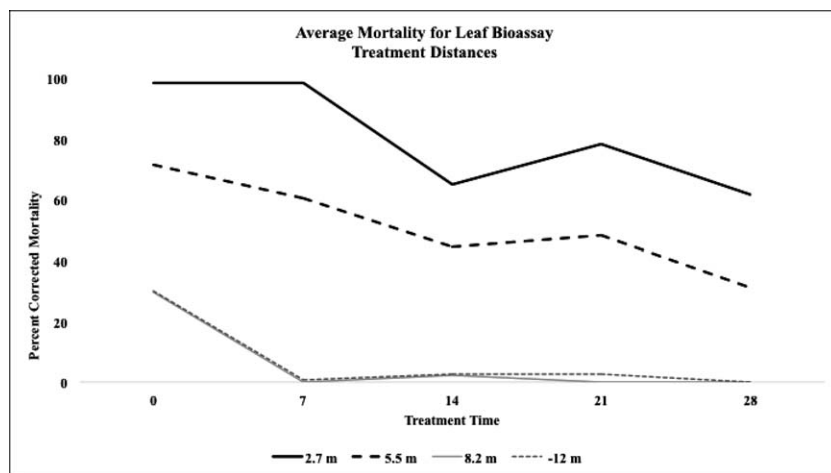


Fig. 3. Leaf bioassay results from average corrected percent adult mosquito (*Aedes aegypti*) mortality data for distances of 2.7 m, 5.5 m, 8.2 m, and -12 m for each posttreatment time (days).

Table 4. Corrected mean percent *Aedes aegypti* (L.) mortality as influenced by distance from sprayer nozzle using a laboratory leaf bioassay encompassing 5 post-bifenthrin application sampling days over 4 wk.^{1,2}

| Distance (m) | Control mortality \pm SE | Treatment mortality \pm SE |
|--------------|------------------------------------|--------------------------------------|
| 2.7 | 2.5 \pm 1.7 a | 80.2 \pm 9.0 a |
| 5.5 | 1.8 \pm 2.9 a | 51.1 \pm 11.2 b |
| 8.2 | 4.5 \pm 2.9 a | 6.4 \pm 4.5 c |
| -12 | 1.9 \pm 1.7 a | 7.2 \pm 5.6 d |
| ANOVA | ($F_{3,39} = 0.86$, $P = 0.47$) | ($F_{3,59} = 20.10$, $P = 0.001$) |

¹ Sampling periods included Day 0, 7, 14, 21, and 28 days after insecticide application. Leaf samples included American sweetgum, American wild grape, and red maple.

² Means followed by the same lowercase letter within a column are not significantly different using a Tukey's multiple means separation procedure. Alpha = 0.05.

switch the control and treatment areas after pretreatment leaf bioassays have confirmed a lack of chemical presence. Having a working internal rather than external tank will allow for the operator to not have an accompanying truck with additional water and chemical if large areas are to be treated, which will increase efficiency of operation and decrease operational costs. According to Hoffmann et al. (2009), truck-mounted equipment is preferable for larger areas to be treated. In this case the operator can be inside the cab while operating the machine remotely, which is an added benefit of this type of machine while covering large areas.

This study adds to the body of literature confirming the efficacy of barrier treatments to block adult mosquitoes from entering human recreational areas. Bifenthrin is a known effective residual insecticide that has been utilized successfully for barrier treatment applications. The machine tested in this evaluation produced excellent vegetation penetration and coverage in an area of dense vegetation with known flood-water and woodland mosquito populations. Such an observation provides an excellent starting point for future studies with this application device.

ACKNOWLEDGMENTS

Thanks to the American LongRay for supplying the equipment. The authors are grateful to K. Gaines, J. McClure, C. Waits, and the NECE staff for their technical help. We thank the University of Florida's veterinary entomology laboratory for providing adult mosquitoes to the study. This is a research report only and does not endorse any of the commercial products involved or mentioned in this report.

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